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SHORT COMMUNICATION

EFFECT OF COLISTIN ON ACTINOMYCIN SENSITIVITY OF *ESCHERICHIA COLI*

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Actinomycin inhibits the multiplication of gram-positive bacteria but not gram-negative bacteria. The mechanism of this action is known to be that it inhibits DNA dependent RNA synthesis (HURWITZ *et al.*, 1962; REICH *et al.*, 1963; LEVINTHAL *et al.*, 1963) presumably by forming a complex with cellular DNA (KAWAMATA and IMANISHI, 1960; RAUEN *et al.*, 1960; KIRK, 1960). The fact that actinomycin inhibits the multiplication of T2 phage in *E. coli* (NAKATA *et al.*, 1960; NAKATA, 1961), however, suggests the possibility of development of actinomycin sensitivity, even in *E. coli*, when the surface is affected by some procedure. As a matter of fact, *E. Coli* cells are rendered actinomycin sensitive when they are converted to spheroplasts by the action of lysozyme and ethylenediaminetetraacetate (EDTA) (MACH and TATUM, 1963; HAYWOOD and SINSHEIMER, 1963), or even by brief treatment with EDTA (LEIVE, 1965).

Some antibiotics are known to affect the permeability of bacteria (FEW and Schulman, 1953; NEWTON, 1953; MIKI, 1960). Colistin, an antibiotic which is a basic peptide (KOYAMA *et al.*, 1950), induces morphological disorganization in bacterial cells (SUGANUMA *et al.*, 1965) as well as leakage of cellular components (KISHIDA *et al.*, 1965). Thus, attempts were made to render *E. coli* sensitive to actinomycin by treatment with a subinhibitory dose of colistin.

The colistin used in this experiment was the sulfate form of a mixture, mainly consisting of colistin A and B, which was a gift from Kayaku Antibiotics Research Co., Ltd., Tokyo. *E. coli*, strain B was grown on Simmons' salt medium, containing 0.3 per cent of glucose with shaking at 37°C.

At the logarithmic phase of growth, the bacterial cells were harvested, washed and then the experiments were carried out by distributing 8 ml portions of bacterial cell suspension containing 5×10^8 cells/ml of Simmons' medium containing 0.3 per cent glucose in L-shaped culture tube. Then 1 ml of colistin and 1 ml of actinomycin D solution were added to give a final volume of 10 ml. The tubes were incubated in a water bath at 37°C and rocked. The growth of bacteria was measured turbidimetrically at 660 mμ with Beckman type, Hitachi Spectrophotometer. The content of DNA was estimated by Burton's method (BURTON, 1956) and RNA by Mejbaum's method (MEJBAUM, 1939).

As shown in Fig. 1a, the growth of *E. coli* is influenced neither by 0.5 μg/ml of colistin nor 100 μg/ml of actinomycin D. When 0.5 μg/ml of colistin and 10 or 100 μg/ml of actinomycin

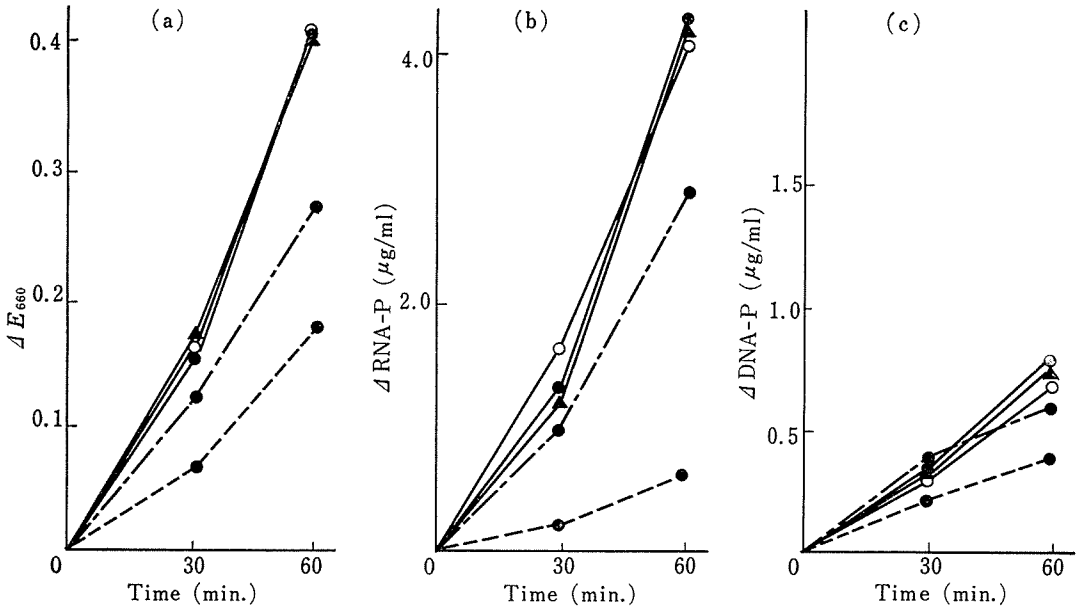

FIGURE 1 Effects of colistin and actinomycin D alone and together on growth (Fig. 1a) and synthesis of RNA (Fig. 1b) and DNA (Fig. 1c) in *E. coli* strain B.

●———● Control
○———○ 100 μg/ml of actinomycin D
▲———▲ 0.5 μg/ml of colistin
●—·—● 10 μg/ml of actinomycin D + 0.5 μg/ml of colistin
●------● 100 μg/ml of actinomycin D + 0.5 μg/ml of colistin

D were added together, the growth of bacteria was slightly inhibited. The net increase of RNA, however, was markedly inhibited by 100 μg/ml of actinomycin D in the presence of 0.5 μg/ml of colistin although no inhibition was observed with either material alone(Fig. 1b). The content of DNA was not lowered appreciably by this combination (Fig. 1c). These results reflect as ensitivity of *E. coli* to actinomycin D evoked by colistin.

Next the effects of actinomycin D on messenger RNA (mRNA) synthesis in *E. coli* were investigated. A culture of *E. coli*, strain B in the logarithmic growth phase was exposed to 0.05 μc/ml of ^{14}C-uracil (Sp. Act. 4.8 mc/mmole) for 60 seconds at 37°C. RNA was extracted with phenol at 4°C in the presence of 0.5 per cent sodium dodecylsulphate in 1 M acetate buffer, PH 5.4. Then the RNA preparation was fractionated by centrifugation on a sucrose gradient.

Figs. 2a-e show the OD and radioactivity profiles thus obtained. Most of the radioactive material sediments as an apparently homologous fraction with a sedimentation coefficient between the 16 S peak and soluble RNA. This rapidly labeled material was identified as mRNA. With 100 μg/ml of actinomycin D alone or 0.5 μg/ml of colistin alone, a pattern similar to that of an uninhibited control culture was obtained (Figs. 2a to c). when 0.5 μg/ml of colistin together with 10 or 100 μg/ml of actinomycin D was added to the culture 5 minutes before labeling, a great decrease in mRNA was observed (Figs. 2d and e).

The results presented here indicate that there are advantages in studying the effect of

actinomycin D on *E. coli*, because the effect of colistin occurs in ordinary growing medium soon after the addition of the antibiotic. This actinomycin sensitivity might be attributed to the effect of colistin on the bacterial cell surface.

Studies on the mechanism of the production of sensitivity to actinomycin by this antibiotic are in progress.

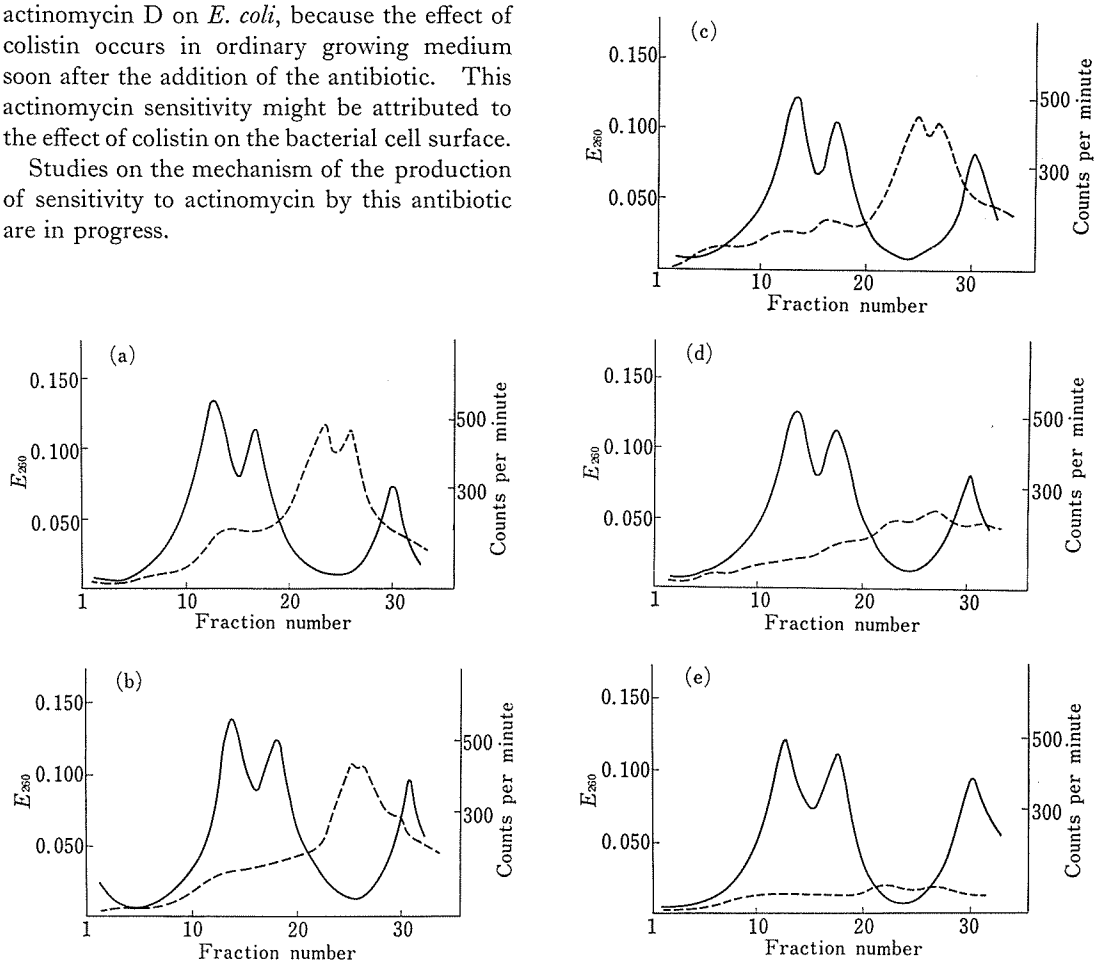

FIGURE 2 Effect of colistin on actinomycin D inhibition of messenger RNA formation in *E. coli* strain B. Sedimentation was carried out in a linear density-gradient of sucrose (5 to 20%) in 0.01 M acetate buffer (pH 5.0) and 0.1 M NaCl in an RPS 40 rotor (Hitachi 40 P ultracentrifuge) at 37,000 rpm for 5 hours.

Fig. 2a shows the profile of RNA of the control culture without antibiotics. Fig. 2b shows the profile of RNA of the culture containing 100 μg/ml of actinomycin D. Fig. 2c shows the profile of RNA of the culture containing 0.5 μg/ml of colistin. Fig. 2d and Fig. 2e show the profile of RNA of cultures containing 0.5 μg/ml of colistin plus 10 μg/ml or 100 μg/ml of actinomycin D respectively. The continuous line represents the optical density (OD) at 260 mμ and the dotted line shows cpm.

——— OD_{260} ------ cpm

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